The properties of reducing agents released by treatment of *Solanum tuberosum* with elicitors from *Phytophthora infestans*

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Tuber and leaf discs of *Solanum tuberosum* were treated with germination fluid from *Phytophthora infestans* cystospores and with several biotic and abiotic elicitors. The subsequent production of reducing agents was determined by the ability of the various treated tuber and leaf discs to reduce cytochrome *c*. The occurrence of superoxide (O_2^-) among the reducing agents was determined by measuring the reduction of cytochrome *c* in the presence and absence of superoxide dismutase (SOD). Although several of the treatments induced the production of reducing agents, very little superoxide was detected in any of the treatments. Reducing agents were present when the discs were treated with germination fluid. Control experiments revealed that these reducing agents were derived solely from the germination fluid in levels comparable to those found in the absence of tuber or leaf discs. The reducing agents in the germination fluid were found to be large molecules (> 10000 mol. wt units), and their ability to reduce cytochrome *c* was enhanced by boiling or by treatment with NADH. The treatment of tuber discs with arachidonic acid, digitonin, or $AgNO_3$ was shown to induce the production of reducing agents. Nearly identical levels of reducing agents were induced by treatment of tuber discs with fatty acids other than arachidonic acid (oleic, linolenic, and eicosapentaenoic acids). The presence of calcium had very little effect on the induction of reducing agents by tuber discs. When leaf discs were also treated with various elicitors, only digitonin and $AgNO_3$ induced the production of reducing agents. These results do not support the hypothesis that superoxide is involved in the resistance interaction between *S. tuberosum* and *P. infestans*.

INTRODUCTION

We have recently demonstrated that the germination fluid produced by zoospores of *P. infestans* elicited the production of phytoalexins in potato tuber discs [*19*]. Chai & Doke [*5*, *6*] have also reported that a similar germination fluid induced the production of superoxide (O_2^-) by discs of potato leaves. Superoxide has long been known to be involved in the defence of mammalian cells. For example, neutrophils generate superoxide when they are taking up invading microorganisms by phagocytosis [*2*]. The possible involvement of superoxide as a defence mechanism in plants certainly warrants investigation, and this study was undertaken to investigate whether germination fluid

which elicits phytoalexin production [*17*, *19*], also elicits the production of O_2^- or other reducing agents when applied to potato tubers and leaves. We were also especially interested in determining whether arachidonic acid, which was first reported to elicit phytoalexins in potato tubers by Bostock *et al.* in 1981 [*3*], could similarly elicit the production of O_2^- in potato tubers or leaves.

MATERIALS AND METHODS

Materials

Seed potato tubers (*Solanum tuberosum* L. cv. Kennebec or Russett Burbank) were planted in 6-inch clay pots in commercial potting soil and watered three times per week with complete Hoagland's solution, as previously described [*16*]. Plants were grown at 20 °C under continuous illumination (20–25 W m^{-2}) provided by fluorescent growth lights (Philips-Westinghouse Corp., 40 W Agro/Lites). Cytochrome *c*, xanthine, xanthine oxidase, and superoxide dismutase were obtained from Sigma. All other reagents were of the highest purity available commercially.

Preparation and treatment of tuber and leaf discs

Leaves were obtained from plants which were planted weekly (from seed tubers harvested in 1986 and 1987) over a period of 18 months. Leaves (3–5 cm in length from 4- to 5-week-old plants) were rinsed in distilled water, and several discs (1 cm diameter) were cut from each leaf with a brass cork borer. The discs were placed with the abaxial surface down on a piece of Whatman No. 1 filter paper in a plastic Petri dish (100 mm). Thirty microlitres of experimental solution was pipetted onto each leaf disc. Then, a small circular piece (6 mm diameter) of Whatman No. 1 filter paper was placed on the leaf disc to spread the solution evenly over the entire upper surface and prevent it from beading up. After all of the discs (usually 15/dish) had been treated, the Petri dish was covered and incubated in the dark at 25 °C. For the preparation of tuber discs, tubers (cv. Kennebec harvested in 1986 and 1987 and stored at 4 °C for at least 4 months) were rinsed in distilled water, peeled, and several cores were removed from each with a brass cork borer (1 cm diameter). Discs (2 mm thick) were cut from the cores and placed on filter paper in Petri dishes as described above. Tuber discs were treated with various experimental treatments by placing 30 µl on the upper surface of the disc and spreading it evenly with the tip of a disposable plastic microlitre pipette.

Germination of zoospores and preparation of germination fluids

Zoospores were prepared from sporangia of *P. infestans* (race 1, 2) by a technique previously described in detail [*17*, *19*] yielding $2–4 \times 10^6$ motile zoospores ml^{-1}. Zoospores were then encysted and germinated by two different methods in order to compare the level and type of reducing agents released during each process. In the first method [*17*, *19*], motile zoospores (30 ml) were transferred from Petri dishes to large test tubes (200 × 25 mm) and mixed at medium speed on a vortex mixer for 60 s to induce synchronous encystment. Cyst preparations were placed in clean Petri dishes, covered, and allowed to germinate at 14 °C. After 20 h the germination fluid was decanted and filtered through Whatman No. 1 filter paper and then through a 0·45 µm

filter to remove all cysts and cellular debris. The germination fluid was then concentrated with an Amicon model 202 ultrafiltration apparatus with PM-10 membranes. The second method of zoospore germination was essentially that of Chai & Doke [*5*]. Motile zoospores in 300 ml of 1 mM $CaCl_2$, pH 6·0, were placed in a 500 ml Ehrlenmeyer flask, and allowed to encyst and germinate by shaking (100 cycles min^{-1}) at 20 °C for either 5 or 20 h. Samples of germination fluid were removed and filtered and concentrated as described above.

Preparation of lipid emulsions

Emulsions of the various fatty acids were prepared by sonicating 10 mg of each fatty acid in 5 ml distilled water for 2 min with a Sonifier model W185 Cell Disruptor, Branson Co., with a micro tip.

Measurement of reducing agents

After the discs had been incubated with various test solutions, five leaf or tuber discs were removed with forceps and placed in vials containing 3 ml of reaction mixture containing 20 μM cytochrome *c*, 0·1 mM EDTA, and 50 mM potassium phosphate buffer (pH 7·8). The degree of reduction of cytochrome *c* which was caused by the presence of superoxide (O_2^-) was determined by measuring the difference in the rate of reduction of cytochrome *c* in the presence and absence of 100 μg superoxide dismutase (SOD) from bovine erythrocytes in the 3 ml reaction mixture. After 10 min in the reaction mixture, the discs were removed with forceps and the concentration of reduced cytochrome *c* was determined by measuring the absorbance at 550 nm. With the tuber discs there was a problem with turbidity (perhaps starch) in the reaction mixture; this was overcome by filtering the solution in a Pasteur pipette packed with glass wool immediately before reading the absorbance. Three control samples of cytochrome *c* solution were completely reduced by adding sodium dithionite, several grains at a time, until a constant absorbance at 550 nm was reached. This value was used to calculate the nmol of cytochrome *c* reduced. Under these experimental conditions the reduction of 60 nmol of cytochrome *c* in the 3 ml reaction mixture yielded an absorbance increase of $0{\cdot}36 \pm 0{\cdot}01$.

RESULTS

Effect of elicitors on the production of reducing agents by potato leaf discs

Germination fluid was prepared using our recently reported technique [*17*, *19*] and applied to discs prepared from potato leaves (Table 1). Treatment of the leaves with water or germination fluid did not stimulate the ability of the discs to reduce cytochrome *c*. In a previous paper [*19*] we reported that although *P. infestans* germination fluid was unable to elicit the production of phytoalexins by potato tuber discs, concentrated germination fluid (concentrated 50-fold) did elicit the production of rishitin by tuber discs. When concentrated (50-fold) germination fluid was applied to potato leaf discs it did stimulate the ability of the leaves to reduce cytochrome *c*. However, the degree of stimulation was nearly the same at the 0, 1 and 3 h measurements. Experiments which were designed to determine the possible reason for this observation will be presented in a later section of this paper. Three other

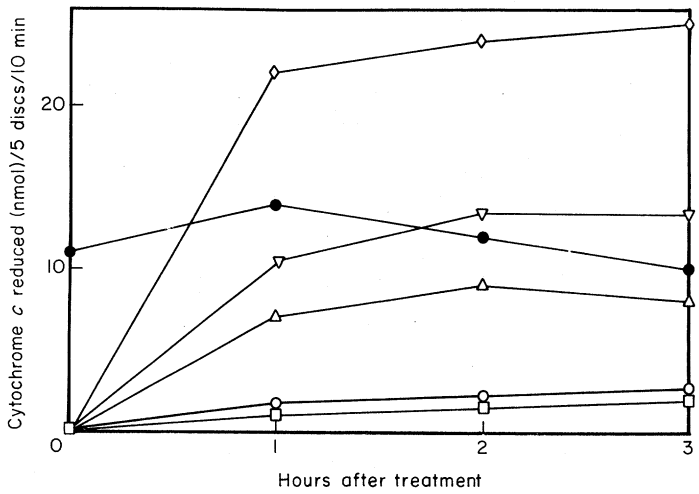

FIG. 1. Time-course study of the effect of various treatments on the rate of cytochrome *c* reduction by discs of potato tuber. To each tuber disc was added 30 µl of the following aqueous solutions: water, only, as a control (□); 2 mg ml^{-1} arachidonic acid (60 µg/disc) (◇); 10 mM $AgNO_3$ (50 µg/disc) (▽); 100 ppm (81 µM) digitonin in 1% ethanol (3 µg/disc) (△); unconcentrated 20 h static germination fluid (○); and 50 × concentrated germination fluid (●).

compounds (arachidonic acid, digitonin, and $AgNO_3$) which have been reported to elicit phytoalexins [*3*, *13*, *14*] were then tested for their ability to elicit the production of reducing agents by leaf discs. Treatment with arachidonic acid caused a very slight increase in the levels of reducing agents. Treatment with digitonin and $AgNO_3$ elicited measurable levels of reducing agents. Although cytochrome *c* continued to be reduced for up to 30 min, reduction was most rapid during the first 10 min, and this time of incubation was chosen for the subsequent experiments. Superoxide dismutase (SOD) was added to the reaction mixture to investigate whether superoxide (O_2^-) was among the reducing agents produced by the germination fluid. Because the presence of SOD had little or no effect on the amount of reducing agent produced, we conclude that very little O_2^- was produced by the germination fluid concentrates. The ability of our SOD to catalyse the breakdown of O_2^- and thus prevent it from reducing cytochrome *c* was verified by using a known O_2^- generating system (xanthine and xanthine oxidase).

Effect of elicitors on the production of reducing agents by tuber discs

In the next experiment (Fig. 1) potato tuber discs were tested with germination fluid and other elicitors, and the levels of reducing agents produced were measured. Treatment of the tubers with water or unconcentrated germination fluid did not stimulate the production of reducing agents. However, as in the previous experiment, reducing agents were produced when the tuber discs were treated with germination fluid concentrated 50-fold. The rate of reduction of cytochrome *c* by discs treated with concentrated germination fluid did not increase during incubation of the discs for 0, 1, 2 or 3 h, and the rate was nearly identical to that reported for the comparable treatment on leaf discs in Table 1. Arachidonic acid, digitonin, and $AgNO_3$ were then tested for their ability to elicit the production of reducing agents by tuber discs (Fig.

TABLE 1

Effect of various treatments on the rate of reduction of cytochrome c *by discs prepared from potato leaves*

Treatments (amount/disc)	nmol cytochrome *c* reduced/5 discs/10 min				
	0 h	1 h		3 h	
	−SOD	−SOD	+SOD	−SOD	+SOD
Control	0·1[a] ± 0·1	0·3 ± 0·2	0·2 ± 0·1	0·3 ± 0·1	0·2 ± 0·1
Germination fluid	0·2 ± 0·1	0·4 ± 0·1	0·3 ± 0·2	0·3 ± 0·1	0·2 ± 0·1
Germination fluid (Conc 50 ×)	8·1 ± 0·9	9·5 ± 1·2	8·7 ± 0·6	8·3 ± 1·0	7·7 ± 0·4
Digitonin, 3 μg	0·2 ± 0·1	2·3 ± 0·3	1·9 ± 0·2	2·9 ± 0·4	2·6 ± 0·2
Arachidonic acid, 60 μg	0·1 ± 0·1	0·6 ± 0·1	0·3 ± 0·1	0·5 ± 0·2	0·2 ± 0·1
$AgNO_3$, 50 g	0·2 ± 0·1	7·3 ± 0·5	5·9 ± 0·3	7·8 ± 0·7	7·1 ± 0·5

Leaf discs were prepared as described and incubated untreated for 1 h. Then, 30 μl of treatment solution was applied to each disc; the discs were incubated for another 0, 1 or 3 h and the levels of reducing agents measured.

[a]Data are the means of at least three determinations ± S.D. This experiment and each of the others were performed at least five times, and for each experiment three analytical determinations were made. The data presented are from one experiment and are representive of the results which were consistently obtained.

1). Each of the three potential elicitors caused an increase in the rate of reduction of cytochrome *c* by treated tuber discs, with the most pronounced increase caused by arachidonic acid, followed by $AgNO_3$, and finally by digitonin.

Control experiments revealed that the stock solutions of arachidonic acid, $AgNO_3$ and digitonin were not capable of reducing cytochrome *c* in the absence of tuber discs (data not shown). Each of the treatments presented in Fig. 1 was also tested for its effect on the rate of reduction of cytochrome *c* in the presence of SOD (data not shown). These experiments revealed that SOD caused only a very small inhibition ($< 10\%$) of the rate of reduction of cytochrome *c* by treated tuber discs, indicating that most of the reducing agents produced in these interactions were not superoxide.

Several laboratories have reported that arachidonic acid and eicosapentaenoic acid were the only fatty acids that elicit the production of phytoalexins in potato tubers [*3*, *4*, *15*]. The next experiment was designed to compare the induction of reducing agents in tuber discs by four common fatty acids. Each of the four fatty acids elicited nearly identical levels of reducing agents (Table 2), and, as with the previous experiments, the levels of O_2^- were very low. Because the levels of reducing agents produced at the 1, 2 and 3 h samplings were nearly identical (Fig. 1 and Table 1), only the 1 h measurement was reported in this experiment and the next.

The next experiment (Table 3) was designed to investigate the effect of Ca^{2+} on the production of reducing agents by treated tuber discs. Recently, Ca^{2+} was reported to stimulate the levels of phytoalexins [*20*] and O_2^- [*5*] produced when potato tubers and leaves, respectively, were treated with elicitors. The presence of 5 mM Ca^{2+} slightly reduced the level of reducing agent produced when tubers were treated with either water, germination fluid, or arachidonic acid (Table 3) and had no apparent influence on the already low level of O_2^- produced. Since we were concerned about the availability of Ca^{2+} when added to the phosphate buffer, because of the low solubility

TABLE 2

Effect of various fatty acids on the rate of reduction of cytochrome c *by discs of potato tubers*

Treatment	nmol cytochrome *c* reduced/5 discs/10 min	
	−SOD	+SOD
Control	3·3[a] ± 0·4	1·9 ± 0·2
Oleic acid, 6 μg	23·1 ± 1·1	21·6 ± 1·5
Oleic acid, 60 μg	56·3 ± 2·4	53·9 ± 2·2
Linoleic acid, 6 μg	24·2 ± 1·8	21·9 ± 1·3
Linoleic acid, 60 μg	54·2 ± 2·2	51·9 ± 2·0
Arachidonic acid, 6 μg	24·2 ± 1·6	23·2 ± 0·9
Arachidonic acid, 60 μg	58·1 ± 2·7	55·3 ± 1·7
Eicosapentaenoic acid, 6 μg	25·7 ± 1·8	23·4 ± 1·1
Eicosapentaenoic acid, 60 μg	59·6 ± 2·8	55·9 ± 2·5

Tuber discs were prepared as described and incubated untreated for 3 h. Then, 30 μl of treatment was applied to each disc; the discs were incubated another hour and the levels of reducing agents were then measured.

[a]Data are the means of at least three determinations ± S.D.

TABLE 3

Effect of Ca^{2+} *on the rate of reduction of cytochrome* c *by discs of potato tubers*

Treatment	Ca^{2+} (5 mM)	nmol cytochrome *c* reduced/5 discs/10 min	
		−SOD	+SOD
Control	0	3·6[a] ± 0·4	2·0 ± 0·2
	5	2·2 ± 0·1	1·7 ± 0·2
Arachidonic acid, 6 μg	0	24·3 ± 1·8	23·1 ± 1·4
	5	20·7 ± 1·1	17·6 ± 0·8
Germination fluid (Conc 50×)	0	11·0 ± 0·4	9·9 ± 0·5
	5	10·4 ± 0·3	9·6 ± 0·3

Tuber discs were prepared as described and incubated untreated for 3 h. Then, 30 μl of treatment was applied to each disc; the discs were incubated another hour and the levels of reducing agents were then measured.

[a]Data are the means of at least three determinations ± S.D.

of calcium in phosphate buffer, another set of experiments was conducted replacing the phosphate buffer with HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid) buffer (50 mM, pH 7·8). It was then necessary to add 0·2 M NaCl to the HEPES buffer to increase the ionic strength enough to prevent the cytochrome *c* from binding to the cell walls of the tuber discs [*18*]. When the experiment reported in Table 3 was repeated using cytochrome *c* buffered with HEPES and including NaCl, the results obtained were nearly identical to those reported in Table 3 (data not shown).

The presence of reducing agents in germination fluid

Because the results of the first two experiments (Fig. 1 and Table 1) indicated that germination fluid itself may contain reducing agents, the next experiment (Fig. 2) was designed to investigate this possibility. The unconcentrated germination fluid did not contain enough reducing agents to cause a measurable reduction of cytochrome *c*.

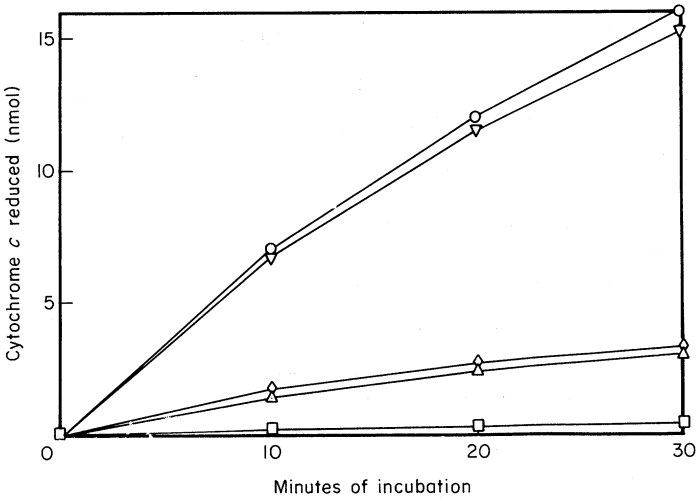

FIG. 2. Time-course study of the reduction of cytochrome *c* by germination fluid from *P. infestans*. The reaction mixture contained 150 µl germination fluid and 3 ml of a mixture containing 20 µM cytochrome *c*, 0·1 mM EDTA, 50 mM potassium phosphate buffer, with and without 100 µg of superoxide dismutase (SOD). The samples were: germination fluid from 20 h static cultures without SOD (□), concentrated (10×) germination fluid without SOD (◇) and with SOD (△), and concentrated (50×) germination fluid without SOD (○) and with SOD (▽).

However, when the germination fluid was concentrated (10- or 50-fold) by ultrafiltration the presence of reducing agents was clearly evidenced. Indeed, the amount of reducing agent was approximately proportional to the degree of concentration in the 10- and 50-fold preparations. This experiment indicates that the reducing agents which were detected when leaf or tuber discs were treated with concentrated germination fluid (Fig. 1 and Table 1) were due mainly to the constituents of the germination fluid and were not generated by the discs. Because the presence of SOD had little or no effect on the amount of reducing agent produced, we conclude that very little O_2^- was produced by the germination fluid concentrates.

In the next experiment (Table 4) germination fluids prepared by our germination technique [*17*, *19*] were compared with fluid from the germination technique that was used by Chai & Doke [*5*] which consisted of gently shaking the sporangia for 5 h to induce encystment and germination. Our 20 h static germination fluid concentrate had a two-and-a-half-fold higher rate of cytochrome *c* reduction than germination fluid prepared by the 4 h shake technique (Table 4). When spores were allowed to germinate for 20 h with shaking, the amount of reducing agents produced was almost identical to that found with the 20 h static technique. This experiment indicates that when spores are germinated for 20 h, using either germination technique, equal levels of reducing agents are produced. When the 20 h static germination fluid was dialysed overnight against 50 mM potassium phosphate (pH 7·8), its subsequent rate of reduction of cytochrome *c* was only slightly less, indicating that the fungal reducing agents are large-molecular-weight compounds (10000 mol. wt units). When the same germination fluid was boiled for 10 min the rate of reduction of cytochrome *c* increased

TABLE 4

The reduction of cytochrome c *by germination fluid from* Phytophthora infestans

Germination fluid (150 µl of 50 × concentrate)	nmol cytochrome *c* reduced 10 min^{-1}	
	−SOD	+SOD
20 h Static	11·0[a] ± 0·2	10·2 ± 0·8
20 h Static, dialysed	9·8 ± 0·4	9·5 ± 0·9
20 h Static, boiled	20·2 ± 1·0	16·0 ± 0·8
20 h Static + 0·5 mM NADH	27·4 ± 1·6	28·1 ± 1·1
4 h Shake	4·3 ± 0·4	4·3 ± 0·5
20 h Shake	11·7 ± 1·1	11·2 ± 0·3

Germination fluids were prepared by a static technique as used in Fig. 1 and previously described by us [17, 19], or by the shaking technique described by Chai & Doke [5]. The other conditions were the same as those for Fig. 2.

[a]Data are the means of at least three determinations ± S.D.

about two-fold. The addition of NADH to the 24 h static germination fluid increased the rate of reduction of cytochrome *c* by more than two-and-a-half-fold. As reported in Fig. 2, the addition of SOD to each of these reaction mixtures had very little effect on the rate of reduction of cytochrome *c*, indicating that O_2^- was not the major reducing agent produced. Only in the case of boiling the fluid from 20 h static germination technique was there a significant reduction in the rate of cytochrome *c* reduction in the presence of SOD.

DISCUSSION

Plants appear to employ many types of defence mechanisms to protect them from pathogenic microbes and insect pests [*7*]. When infected by fungal pathogens, certain cultivars of potato have been shown to produce several types of phytoalexins [*7*]. Despite extensive research, very little is known about the mechanism of recognition of the fungus by the plant, and the initial events leading to phytoalexin production. Several types of elicitors have been prepared from *P. infestans* and shown to cause the production of phytoalexins upon treatment of potato discs. Soluble and insoluble glucans of the fungus [*10*], arachidonic and eicosapentaenoic acids, which are major fatty acid in the membranes and storage lipids of the fungus [*3*], and the germination fluid of the fungus, which contains mostly proteins [*5, 19*] have been shown to be active in this respect. It is not yet known which of these fungal materials, if any, are actually recognized by the plant *in vivo* and trigger a resistance reaction. Recently, Doke's group [*5, 6, 10–13*] published evidence that superoxide may either be released by the plant during the initial recognition of the fungus or fungal elicitor, or may serve as a defence mechanism due to its antimicrobial activity. The antimicrobial activity of superoxide has been elegantly demonstrated in certain mammalian cells [*2*]. Another recent report [*14*] provided evidence that hydroxyl radicals may be involved in the elicitation of phytoalexins in legumes. The results of this study indicate that reducing agents are produced by potato tubers and leaves after they have been treated with certain

elicitors. However, there was very little evidence that appreciable levels of superoxide were among the reducing agents produced in any of our experimental treatments.

This study was undertaken because we were interested in taking a system in which we had previously demonstrated the elicitation of rishitin production by treatment of tuber discs with germination fluid [*19*] and arachidonic acid [*15*] and investigating whether the production of superoxide or other reducing agents correlated with our previously reported values for rishitin production. It was not our intention to try to duplicate the exact conditions of previous reports [*5, 6, 10–13*]. For instance, although others [*3–6*] have reported that tuber slices had to be aged for 12 to 24 h before they would react hypersensitively to infection or elicitor preparations, our laboratory has never found any advantage in "aging" slices for more than 1 to 3 h. Because this shorter period of aging was used in the previous paper [*19*] in which we consistently observed the elicitation of rishitin by germination fluids concentrated 50-fold we decided to continue this practice in the present study. If the production of superoxide is observed only when tissues are subjected to very narrowly defined conditions, and is not observed under conditions which have been shown to elicit the production of phytoalexins, then we question its role in the resistance of potatoes to diseases.

This is the first study which has investigated the possible elicitation of superoxide and other reducing agents by arachidonic acid. Although previous studies have revealed that arachidonic and eicosapentaenoic acids were the only fatty acids that elicited phytoalexins in potato tuber slices [*3, 4, 15*], our results indicate that each of the four unsaturated fatty acids elicited the same level of production of reducing agent by tuber discs (Table 3). Two previous studies have also shown a similar non-specific response by unsaturated fatty acids [*4, 8*]. Addition of either arachidonic acid or linoleic acid to tuber discs elicited the production of comparable levels of ethylene and ethane [*4*]. Davis & Currier [*8*] reported that arachidonic acid and several other fatty acids caused a similar degree of cell death when applied to tuber slices [*8*]. The ability of the four types of fatty acids to elicit the production of reducing agents in the current study may result from the enhancement of leakage of cytosolic reducing agents such as ascorbic acid, which is present at levels of about 0·2 mg g^{-1} fresh weight of potato tubers [*9*].

We believe it is important to point out that the high (0·1–1 μmol min^{-1}) rates of cytochrome *c* reduction which were reported in previous studies with potato tissue [*5, 6, 10–13*] appear to be impossible using the experimental system described by the authors. In the present study, and those of Doke's group [*5, 6, 10–13*] the concentration of cytochrome *c* in the reaction mixture was 20 μM, so the total amount of cytochrome *c* in a 3 ml reaction mixture was 0·060 μmol (60 nmol). Thus, the maximum rate of reduction of cytochrome *c* that could be measured by their experimental system is actually 0·06 μmol per 5 discs per 10 min. These calculations reveal that the rates of reduction of cytochrome *c* reported in the previous studies [*5, 6, 10–13*] were many-fold higher than could have been measured under the experimental conditions described. We assume that the rates of cytochrome *c* reduction which were probably observed in the previous studies were actually of the order of 0·1–6 nmol min^{-1}, similar to those observed in this study. Our observed increase in the absorbance of cytochrome *c* via dithionite reduction is consistent with the reported molar extinction coefficient for cytochrome *c* which was used by Chai & Doke [*6*]. The involvement of superoxide in disease resistance is an attractive hypothesis, and further evidence for its involvement

was recently reported with rice leaves in relation to blast resistance [*1*]. However, our results suggest that further experimentation is required to establish the involvement of superoxide in the potato–*Phytophthora infestans* interaction.

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